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PRINCIPAL INVESTIGATOR: Mengna Xia
Hanli Liu

CONTRACTING ORGANIZATION: The University of Texas at Arlington
Arlington, TX 76019

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Mengna Xia
Hanli Liu

E-Mail: hanli@uta.edu

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Objective/Hypothesis: By monitoring global and local vascular oxygenation and tissue oxygen tension in breast tumors under HBO exposure with several different gas interventions, we wish to prove the following two hypotheses: that 1) the combination of HBO and hyperoxic intervention can largely improve breast tumor oxygenation, and that 2) tumor oxygenation remains elevated for a period of time even after HBO exposure, which may provide a unique treatment window to enhance radio-sensitivity. Specific Aims: Aim 1: to determine the absolute values of oxygenated hemoglobin concentration, [HbO2], and hemoglobin oxygen saturation, SO2, in solid breast tumors from the NIRS measurements. Aim 2: to investigate vascular oxygenation and tissue oxygen tension of breast tumors under continuous normobaric and hyperbaric oxygen exposures with several gas interventions, using both a single-channel NIRS system and 3-channel FOXY pO2 system simultaneously. Aim 3: to investigate global and local dynamics of tumor vascular [HbO2] and tissue pO2 of breast tumors immediately after HBO exposure using both three-channel NIRS and 19F MR EPI imaging simultaneously.

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Technology Development, Near infrared spectroscopy, Hyperbaric Oxygen Treatment, Tumor Therapy Planning and Prognosis, Tumor Physiology Monitoring.

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# 2006-2007 ANNUAL PROGRESS REPORT

This report presents the specific aims and accomplishments of our breast cancer research project during the last year of funding sponsored by the US Department of the Army. It covers our activities from May 1, 2006 to April 30, 2007.

## 1. Introduction

The overall goal of this research project is to apply the multiple monitoring techniques, i.e. Near infrared spectroscopy (NIRS), FOXY oxygen sensor and $^{19}F$ MR EP imaging of Hexafluorobenzene (HFB), to prove the following hypotheses: combination of hyperbaric oxygen (HBO) intervention can significantly improve breast tumor oxygenation, and that tumor oxygenation remains elevated for a substantial period of time even after HBO exposure, which may be a novel approach to enhance radiosensitivity or chemotherapy. If our hypotheses are proven to be true, this study will lead to an optimal intervention plan to improve tumor oxygenation and to determine an optimal time interval after HBO decompression for radiotherapy. Such a novel approach will largely enhance the efficiency of non-surgical therapies for breast tumor treatment and provide a novel prognostic tool for clinical practice. This study will also provide a better understanding of tumor vasculature and tissue oxygen dynamics and spatial heterogeneity under HBO exposure.

***The project has three specific aims:***

***Aim 1:*** to determine the absolute values of oxygenated hemoglobin concentration, [$HbO_2$], and hemoglobin oxygen saturation, $SO_2$, in solid breast tumors from the NIRS measurements.

***Aim 2:*** to investigate vascular oxygenation and tissue oxygen tension of breast tumors under continuous normobaric and hyperbaric oxygen exposures with several gas interventions, using both a single-channel NIRS system and 3-channel FOXY $pO_2$ system simultaneously.

***Aim 3:*** to investigate global and local dynamics of tumor vascular [$HbO_2$] and tissue $pO_2$ of breast tumors immediately after HBO exposure by using both three-channel NIRS and $^{19}F$ MR EP imaging simultaneously.

In our earlier studies, we have accomplished Aims 1 and 2. Based on the studies performed for Aims 1 and 2, we have recognized that it would be very practically useful if hyperbaric oxygen exposure could enhance therapeutic effects for chemotherapy. So, we modified Aim 3 accordingly, as given below:

***Aim 3: to investigate (1) whether HBO could enhance the therapeutic efficiency of malignancy when used as a chemotherapeutic adjuvant of an anticancer agent (doxorubicin, DOX) in mammary carcinomas of rat model, and (2) the feasibility of NIRS to monitor tumor hemodynamic changes resulting from the therapeutic effect of DOX on vasculature.***

For the last year, we have focused on achieving Aim 3, as reported below.

## 2. Body of the Report

Basically, the target of Aim 3 is to study "Tumor vascular oxygenation monitored by NIRS in rats with hyperbaric oxygen intervention in combination with doxorubicin treatment."

### 2.1 Introduction

Doxorubicin (DOX) is one of the most widely used broad-spectrum anticancer agents [1]. However, its clinical utility is limited, because this agent produces a chronic and dose-related cardiomyopathy as its principal side effect. Therefore, it is desirable to achieve better chemotherapeutic effect with lower dosage of the agent. It is well accepted that hypoxic tumor is resistant to radiotherapy and some chemotherapy agent [2,3,4,5]. To overcome hypoxia, a variety of approaches have focused on improve oxygen delivery via oxygen-enriched gases or blood substitutes [6,7,8,9,10]. Hyperbaric oxygen was believed to improve tissue oxygenation greater than normabric oxygen because it increased oxygen tension and oxygen delivery to tissue independent of hemoglobin[11]. HBO, as a chemotherapy adjuvant in tumor treatment rather than stand alone treatment, is believed to increases cellular uptake of some chemotherapy agents and the susceptibility of cells to these agents. It has been demonstrated that HBO can increase the susceptibility of malignant cells to destruction with taxol [12], doxorubicin [13, 14] and 5-FU [15,16].

The influence of tumor oxygenation on treatment outcome has stimulated various techniques to monitor or estimate tumor oxygenation. These include microelectrodes, optical reflectance, electron paramagnetic resonance (EPR), magnetic resonance imaging (MRI) and nuclear medicine approaches, as reviewed previously [17]. As each approach has their own strength, some are highly invasive. Since its introduction in 1970s [18], Near Infrared Spectroscopy has been increasingly applied to study tissue oxygenation status non-invasively. Near infrared light can easily penetrate biological tissue, and allow for detection of specific light-absorbing chromophores in human in vivo, such as oxygenated and deoxygenated hemoglobin, water and lipid [19]. it has been used extensively for quantitative measurements of cerebral oxygenation [20] and blood oxygenation in muscles *in vivo* [21], and more recently, tumor vascular oxygenation with respect to interventions [22]. NIRS currently lack of spatial resolution, and thus, the utility of global measurement require validation, given the well-documented heterogeneity of tumor. In this regard, Xia *et al* [22] compared the spatially averaged measurement of relative tumor oxygen saturation ($_SO_2$) using NIRS with the local $pO_2$ measured by MRI. The sensitivity and specificity analysis suggests that NIRS may identify clinically relevant hypoxia, even when its spatial extent is below the resolution limit of the NIRS technique. Kim *et al* demonstrated that NIRS may be used as an effective tool to monitor tumor hemodynamic change induced by some vascular disrupting agent [23,24].

These studies were designed to investigate 1) whether HBO could enhance the therapeutic efficiency of malignancy when used as a chemotherapeutic adjuvant of doxorubicin in mammary carcinomas of rat model, and 2) the feasibility of NIRS to monitor tumor hemodynamic changes resulting from the therapeutic effect of DOX on vasculature.

## 2.2 Materials and Methods

### *2.2.1 Animal and Tumor models*

Healthy female Fischer 344 rats aged 5 to 6 months were obtained from Harlan Sprague-Dawley (Indianapolis, IN). They were housed for at least a week to acclimatize and for monitoring of health before inclusion in the study. Mammary carcinomas 13762NF were implanted in the dorsum of female Fischer 344 rats weighing ~200g. Tumor volume was estimated by the formula of 6/pi *(LxWxH), Tumor diameter was measured in orthogonal axes (L,W, H). Tumor size and body weight were monitored every other day after therapeutic interventions. All animal protocols were approved by Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center and University of Texas at Arlington.

### *2.2.2 Drug preparation and dose*

Doxorubicin Hydrochloride was purchased from Sigma Aldrich, Inc. It was made into a solution by dissolving with saline. A single dose of DOX (2mg/kg body weight) was administrated by tail vein. The dose of doxorubicin given was the usual chemotherapeutic dose, which would not cause cardio-toxicity [13].

### *2.2.3 Experimental procedure*

Following tumor establishment (~1 cm diameter), rats were randomly assigned to one of three groups according to different therapeutic strategies: a) DOX (n=5), b) HBO + DOX (n=5). C) control group (n=2) with saline injection. Rats were anesthetized with the mixture of ketamine hydrochloride (0.15 ml; 100mg/ml; Aveco, Fort Dodge, IA) and xylazine via i.p. After tumor hair was shaved to allow better optical contact for NIR light transmission, the rat was placed on its side in the hyperbaric chamber. Probes of SSDRS were fixed securely on the tumor of rat, and then SSDRS monitored tumor vascular oxygenation while the rat was subjected to therapeutic interventions.

DOX group was given 0.4 mg/ml doxorubicin solution intravenously after the respiratory intervention of air -$O_2$ - air, and then were exposed to air – $O_2$ - air after DOX injection. HBO + DOX group was exposed to gas intervention in a sequence of air-$O_2$ - HBO (30 min) prior to intravenously administration of 0.4 mg/ml doxorubicin solution, and then exposed to air - $O_2$ – air. Control group was subjected to the respiratory intervention of air -$O_2$ - air, saline solution intravenous injection and then were exposed to air – $O_2$ – air. To control the timing of DOX injection, an IV butterfly catheter was inserted into rat tail vein and fixed securely with tape before the rat was put into the chamber. The syringe filled with DOX solution was connected to the catheter with heparin only before DOX injection, in order to avoid the possible precipitation caused by the incompatibility of DOX and heparin [25].

### *2.2.4 Spectrometer for monitoring the disturbance of DOX on the absorption of tissue phantom*

Because the reddish color of doxorubicin, it is reasonable to consider the bolus injection of doxorubicin may change tissue absorption. A UV/VIS spectrometer Lambda 20 (PerkinElmer Inc., Waltham, MA) was used to detect the absorption change resulting from the addition of DOX into tissue phantom, in order to verify the acute effect of DOX bolus injection on tissue absorption. A tissue phantom is composed of 100 μl sheep blood mixed with phosphate buffer solution with total volume of 3.5 ml and total hemoglobin concentration of 7.1 g/L, which is in the range of total hemoglobin concentration in tissue [26]. The Hb concentration of tissue phantom was measured by Co-oximeter (Instrumentation Lab, Ramsey, MN). Since animal's total blood volume is 10% of its body weight, total blood volume = 0.2 Kg x 10% = 20 ml, with the known body weight of ~ 0.2 Kg of rats. 0.17 ml DOX solution with the concentration of 0.4 mg/ml was added into the tissue phantom. Accordingly, the volume ratio of DOX to tissue phantom is proportional to the ratio of 1 ml 0.4 mg/ml DOX to 20 ml total blood volume in rats. According to the Lambert Beer Law, the absorption in 750nm and 830nm are

$$A^{750}(0) = \varepsilon_{Hb}{}^{750}[Hb] + \varepsilon_{HbO_2}{}^{750}[HbO_2] \quad (2.1)$$

$$A^{750}(1) = \varepsilon_{Hb}{}^{750}[Hb] + \varepsilon_{HbO_2}{}^{750}[HbO_2] + \varepsilon_{DOX}{}^{750}[DOX] \quad (2.2)$$

$$A^{830}(0) = \varepsilon_{Hb}{}^{830}[Hb] + \varepsilon_{HbO_2}{}^{830}[HbO_2] \quad (2.3)$$

$$A^{830}(1) = \varepsilon_{Hb}{}^{830}[Hb] + \varepsilon_{HbO_2}{}^{830}[HbO_2] + \varepsilon_{DOX}{}^{830}[DOX] \quad (2.4)$$

where $A^{750}$ (0) and $A^{750}$ (1) represent the absorption at 750 nm in tissue phantom without DOX and with DOX, respectively. $A^{830}$ (0) and $A^{830}$ (1) represent the absorption at 830 nm in tissue phantom without DOX and with DOX, respectively.

*2.2.5 Steady-state diffuse reflectance spectroscopy (SSDRS) for measuring changes in tumor vascular oxygenation ($\Delta HbO_2$)*

A broadband diffuse reflectance spectrometer was used to acquire reflectance spectra from tumor tissue. Briefly, continuous wave (CW) light from a 20 W tungsten-halogen light source (HL-2000HP, ocean optics, FL) is coupled into a 2.6-mm core diameter fiber optic bundle, the distal end of which is placed in physical contact with the surface of the tumor. After being scattered in the tumor tissue, the transmitted light is collected by a 1-mm core diameter detection fiber, the end of which is coupled to a hand-held spectrometer (USB2000, Ocean optics, FL). The broadband light diffuse spectrometer provides reflectance spectra from 400 to 900 nm.

According to the modified Beer-Lambert law, changes of oxy- and deoxy-hemoglobin concentration, $\Delta[HbO_2]$ and $\Delta[Hb]$, can be derived from the measured amplitudes at two wavelengths (750nm and 830nm), by using extinction coefficients of oxy- and deoxy-Hb published by Cope [27], as given in Equations. (2.5) and (2.6):

$$\Delta[HbO_2] = \frac{-1.532\log(\frac{A_b}{A_t})^{750} + 1.753\log(\frac{A_b}{A_t})^{830}}{DPF \cdot d} \quad (2.5)$$

$$\Delta[Hb] = \frac{1.758 \log(\frac{A_b}{A_t})^{750} - 0.92 \log(\frac{A_b}{A_t})^{830}}{DPF \cdot d} \quad (2.6)$$

where $A_b$ is the baseline amplitude, $A_t$ is the transient amplitude during the intervention, and $d$ is the direct source-detector separation. *DPF* (differential path-length factor) is a tissue-dependent parameter and defined as the ratio between the optical path length and the physical separation between the source and detector.

## 2.3 Results

### *2.3.1 Disturbance of DOX on the absorption of tissue phantom*

There is absorption difference between tissue phantom with and without DOX, as shown in Figure 2.1. However, the differences are relatively small. Indeed, the relative changes of absorption in wavelength of 750nm and 830nm are $\frac{A^{750}(1) - A^{750}(0)}{A^{750}(0)}$=4.85% and $\frac{A^{830}(1) - A^{830}(0)}{A^{830}(0)}$=4.80%, respectively. In order to investigate the disturbance of DOX on calculating oxygen saturation, absorption spectrum profiles between 700 and 900 nm were normalized by absorption values at the wavelength of 700 nm. The normalized spectra cover the wavelengths we utilized for calculating hemoglobin concentration. As shown in figure 2.2, profiles of normalized absorption spectrum in NIR range appeared to be overlaid.

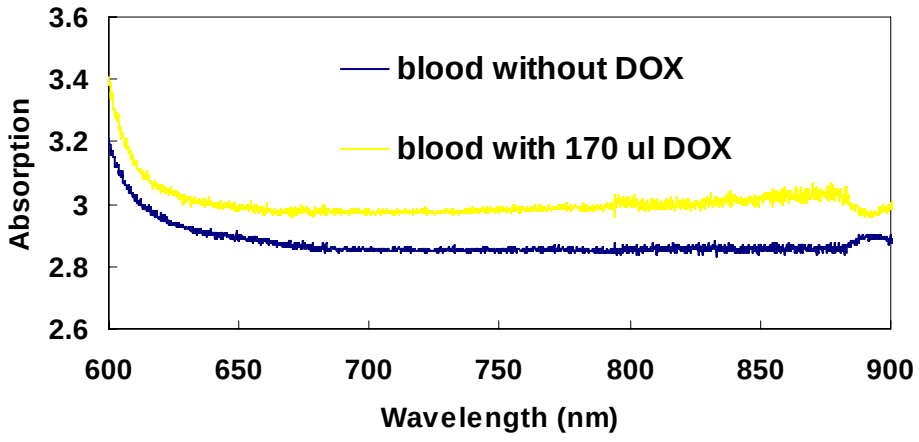



Figure 2.1 Absorption spectra of tissue phantoms with (yellow curve) or without 170 μl DOX (blue curve). The unit for absorption is arbitrary unit.

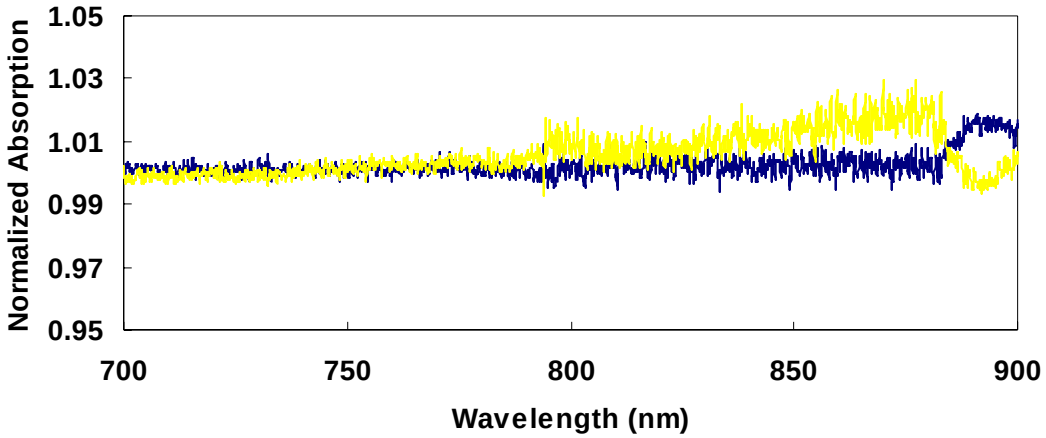
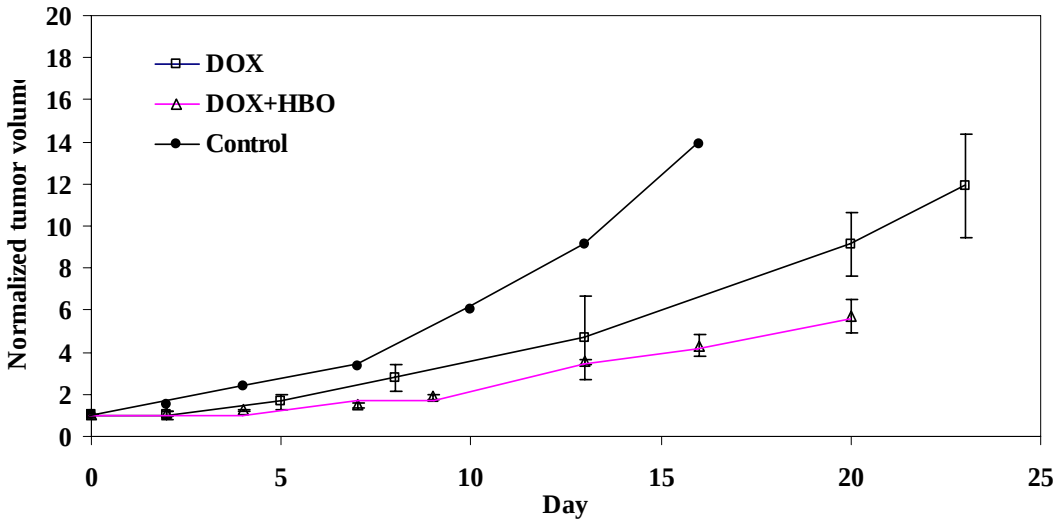



Figure 2.2 Normalized absorption spectra of tissue phantoms with (yellow curve) or without 170 μl DOX (blue curve) in the wavelength range of 700 ~ 900 nm.

### *2.3.2 Changes in Tumor volume and body weight during chemotherapy*

Tumor volume and body weight were monitored before and after DOX treatment to examine the tumor response. Changes in tumor volume and body weight were normalized by the values at day 0 (before DOX administration).

a)



b)

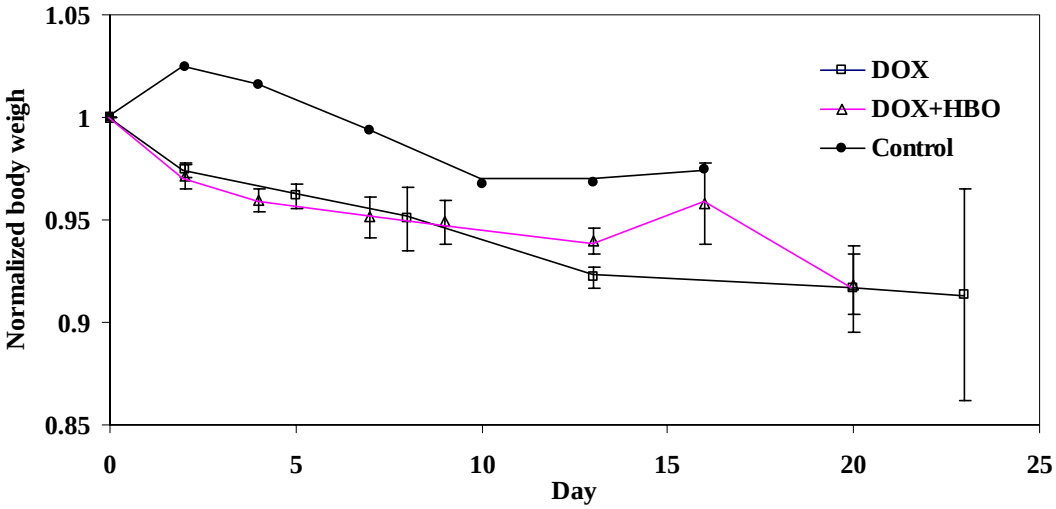



Figure 2.3 Normalized (a) tumor volume and b) body weight in rats with saline injection ( ● ), DOX treatment ( □ ) and HBO + DOX treatment ( Δ ).

Tumors in the control group grow significantly faster than tumors with treatment in the other groups ($p<0.05$), as shown in Figure 2.3a. Tumors with combined treatment of HBO and DOX grow significantly slower than those with DOX treatment except for the first two days after treatment ($p<0.05$). Basically, there is no significant difference for tumor volume in HBO + DOX and DOX groups on the 2nd day after treatment. However, significant differences are observed starting from 5th day after treatment between the DOX treated and control group ($p< 0.05$). Regarding body weight loss, figure 2.3b indicated that rats in DOX group and DOX + HBO group had significant and continuous body weight loss after day 0. Rats in control group gained weight at day 2, and started to lose weight after day 4, and then kept constant body weight thereafter.

*2.3.3 Vascular hemodynamic changes of rats in DOX group*

Values of $\Delta[HbO_2]$ of rats in DOX group showed increases with values in a range of 0.07 ~ 0.12 (mM/DPF), when the gas switched from air to oxygen before DOX injection. When the gas was switched from air to oxygen after DOX administration, the maximal amplitude of $\Delta[HbO_2]$ increased with values in a range of 0.02 ~ 0.04 (mM/DPF), which is significantly less than the increase achieved with $O_2$ inhalation before DOX administration, as shown in figure 2.4. We also noticed the signal changes during DOX i.v. injection.

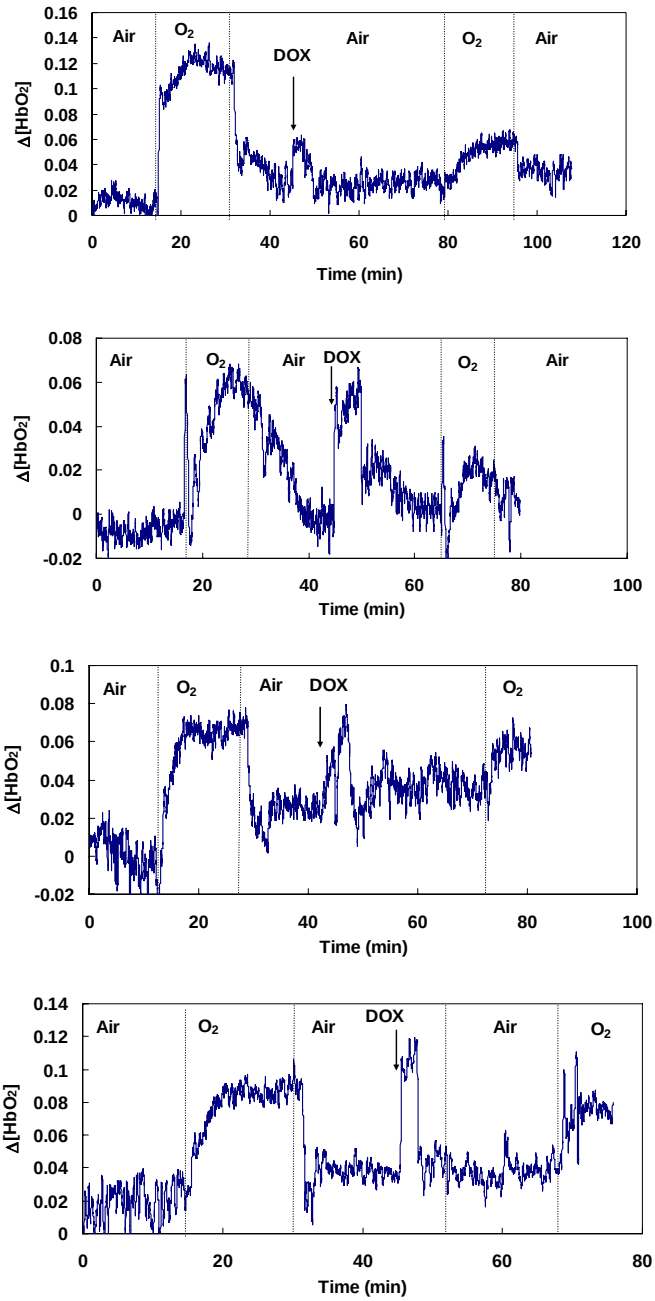



Figure 2.4 Time profile of $\Delta[HbO_2]$ of tumors (1~4) in DOX group when the rat was under gas intervention. The unit for $\Delta[HbO_2]$ is mM/DPF. $\Delta[HbO_2]$ of the 5th rat was discarded because of misplaced gas mask during the experiment due to the movement of the rat.

*2.3.4 Vascular hemodynamic changes of rats in DOX +HBO group*

It shows a stable baseline in $\Delta[HbO_2]$ when rats were inhaling air. $\Delta[HbO_2]$ increase immediately in the first few minutes and more gradually afterwards, as shown in figure 2.5. When the rats were exposed to hyperbaric oxygen, $\Delta[HbO_2]$ has a further increase until reaching a stabilized value. DOX i.v. injection immediately after HBO caused some fluctuation of signal, but stabilized when the injection is finished. $\Delta[HbO_2]$ has a stable baseline but with values greater

than air inhalation before HBO when the rats were breathing air after HBO. Similar to DOX group, the maximal amplitude of $\Delta[HbO_2]$ achieved with $O_2$ inhalation prior to the DOX administration is significantly greater than that achieved with $O_2$ inhalation after DOX administration.

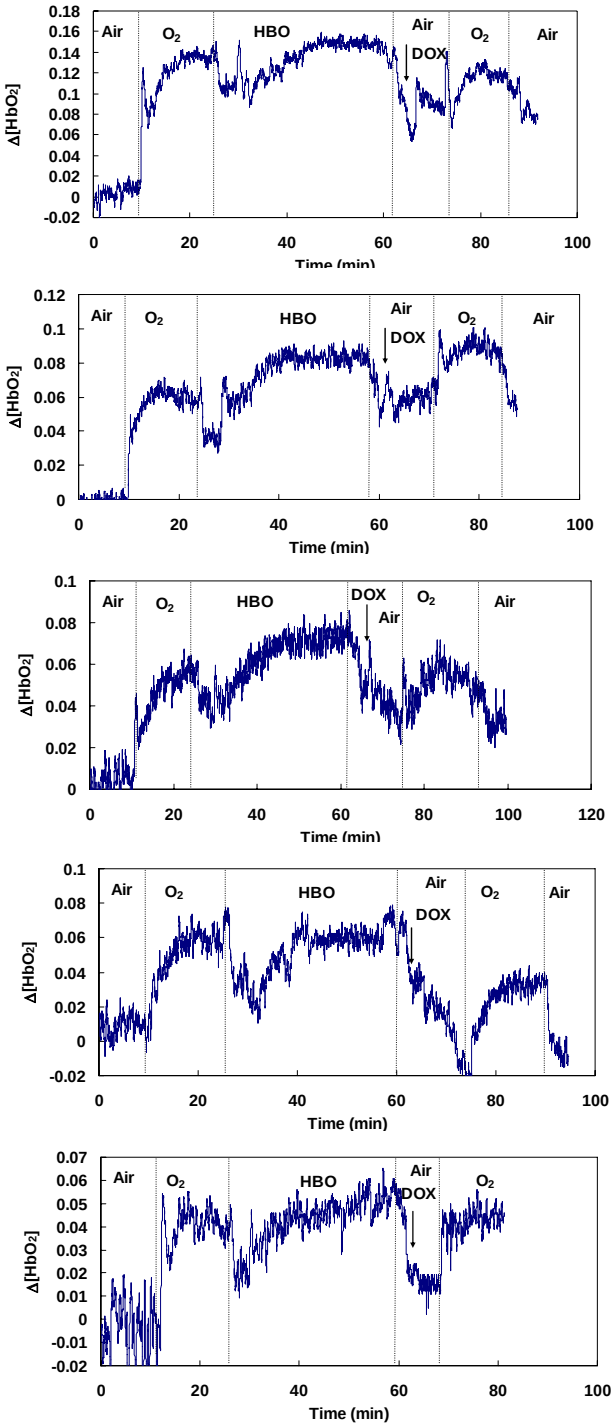



Figure 2.5 Typical time profile of $\Delta[HbO_2]$ of tumors in DOX + HBO group (n=5) when the rat was under gas intervention.

*2.3.5 Vascular hemodynamic changes of rats in control group*

$\Delta[HbO_2]$ have stable baseline values when rats were breathing air, as shown in figure 2.6. $\Delta[HbO_2]$ increased when the gas was switching to oxygen, and decreased when the gas was switched back to air, as expected. Different from $\Delta[HbO_2]$ in DOX treated group, the change of amplitude in $\Delta[HbO_2]$ when the gas switched from air to oxygen after saline injection is similar to the change due to gas intervention before injection in both rats.

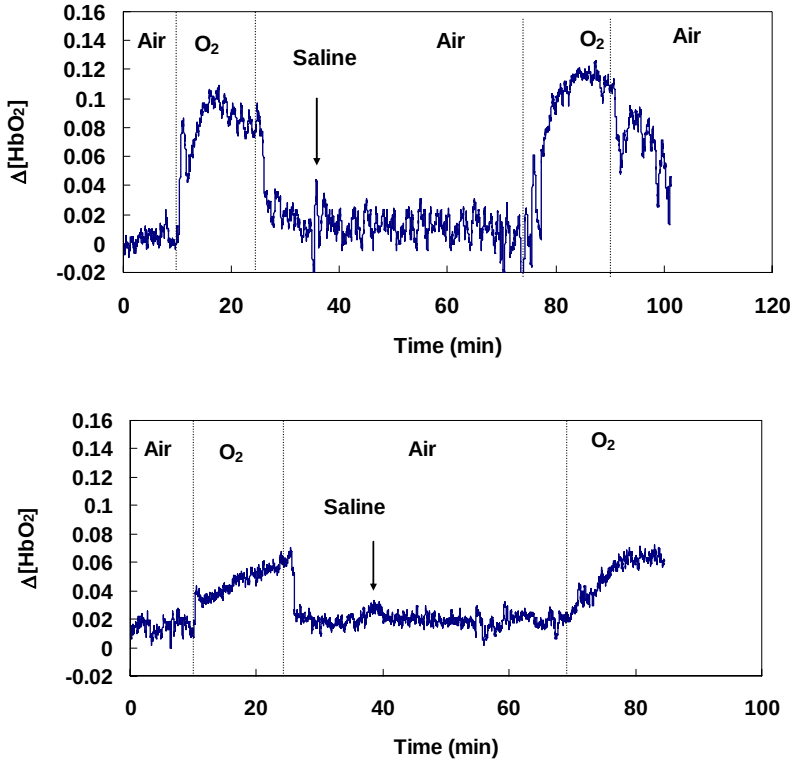



Figure 2.6 Time profile of $\Delta[HbO_2]$ of tumors in control group (n=2) when rats were subjected to gas intervention.

## 2.4 Discussion and Conclusion

In our study, we randomized breast tumors into three groups: group with DOX injection, group with DOX injection following HBO exposure at 2 atm, control group with saline injection. The result showed that tumors in HBO + DOX group grow significantly slower than those with DOX alone. HBO enhanced chemotherapeutic response of mammary carcinoma NF13762 to DOX *in vivo* reflected by slowing down the tumor growth after treatment.

Resistance to chemotherapy is common in hypoxic tumors. HBO may help overcome chemotherapy resistance by improving both tumor perfusion and cellular sensitivity. Improving tumor oxygenation and vascularization may increase drug delivery. This has been shown

experimentally and in nude mice with human epithelial ovarian cancer treated with cisplatin [28]. Reactive oxygen species (ROS), or free radicals are by-product of aerobic respiration and cellular metabolism and induced by oxidative stress during hypoxia (oxygen deficiency), reperfusion or hyperoxia (excess oxygen). ROS, at low levels, assist tumor growth but become toxic at high levels. This can be explained by the "threshold effect" whereby ROS reach a level beyond which the antioxidant capacity is inundated, resulting in irreversible damage and apoptosis [29,30]. One of the mechanisms of action of doxorubicin is production of ROS. By increasing ROS level, HBO push ROS levels past the threshold level, and thus enhanced the ROS-localized effects of doxorubicin [31,32]. Another mechanism of HBO is to push cell to enter a proliferate stage, thus sensitizing them to radiotherapy and some chemotherapy by improving oxygenation. It has been showed that HBO enhanced the chemotherapeutic effects of doxorubicin in an experimental model of pulmonary sarcoma [13]. HBO stimulated proliferation of an MCA-2 metastatic lung tumor cell line and induced cells to enter the replicating cycle compared to cells left at ambient pressure [13]. Other studies found that HBO increased the ratio of prostate cancer cells *in vitro* accumulating in $G_2$/M phases from the $G_0$ arrest phase [33]. Generally speaking, HBO therapy in combination with chemotherapy may be justified by the following: 1) improved oxygenation improves drug delivery to hypoxic regions in the tumor; 2) increasing intratumoral ROS levels beyond the threshold may induce tumor destruction; 3) improved oxygenation may also cause cell to enter a proliferate stage, thus sensitizing them to radiotherapy and some chemotherapy; 4) HBO may remove hypoxia stimulus that drives angiogenesis.

Even though the DOX dosage is reported to cause minimal cardiotoxicity, the result showed that changes in amplitudes of $\Delta[HbO_2]$ before DOX administration were much greater than the change after treatment in rats of DOX group and DOX + HBO group. It is likely that the amplitude difference results from the known cardiotoxicity reaction, the major side effect of DOX. It has been suggested that cardiac dysfunction induced by DOX resulted from the imbalances of the circulatory system such as decreases in blood pressure or the direct effects on vascular wall [34]. During the course of i.v. injection of DOX, the vasculature was exposed to high levels of DOX, and *in vitro* studies have suggested that DOX acutely induces vascular smooth muscle to release $Ca^{2+}$ from its intracellular storage site and causes direct vasoconstrictor [35] and vasodilator effects [36]. Furthermore, the combination of doxorubicin and HBO would also be expected to enhance the agent's cardiotoxicity because of the toxicity to cardiomyocytes of HBO. Therefore, the clinical addition of HBO to doxorubicin may not change the risk-benefit ratio of the agent. NIRS, in turn, may provide a novel approach to monitor the cardiotoxicity of treatment, which may leads to an optimized therapeutic plan to minimize the side effect of treatment. We also noticed the signal fluctuation in $\Delta[HbO_2]$ during DOX injection in DOX group. DOX solution is orange-red, so it is likely that DOX bolus injection would cause the absorption change of tumor tissue in NIR range. We measured and compared the spectra of tissue phantoms before and after adding DOX, to examine the disturbance of DOX injection on the signal of $\Delta[HbO_2]$. There were absorption differences when comparing both absorption spectra (Figure 2.1). However, the normalized spectra appeared to be overlaid (Figure 2.2). Therefore, it implied that DOX would affect total hemoglobin concentration, rather than oxygen saturation.

## 3. Key Research Accomplishments and Reportable Outcomes

**Publications in peer-reviewed journals:**

1. **Mengna Xia**, Vikram Kodibagkar, Hanli Liu and Ralph Mason, "Tumor oxygen dynamic measured simultaneously by near infrared spectroscopy and $^{19}F$ magnetic resonance imaging in rats", Physics in Medicine and Biology,51: 45-60 (2006) .

2. Jae Kim**, Mengna Xia,** Hanli Liu, **"**Extinction coefficients of hemoglobin for near-infrared spectroscopy of tissue", IEEE Engineering in Medicine and Biology magazine, 24: 118-121 (2005).

3. Yueqing Gu, Wei R. Chen, **Mengna Xia**, Sang W. Jeong, and Hanli Liu, "Effect Of photothermal therapy On breast tumor vascular contents: non-invasive monitoring by near infrared spectroscopy", Photochemistry and Photobiology, 81: 1002-1009 (2005).

**Proceeding papers and presentations:**

1. **Mengna Xia,** Benjamin Levine, Ralph Mason, Hanli Liu, "Simultaneous monitoring of tumor vascular oxygenation and tissue oxygen tension under hyperbaric oxygen exposure", in Biomedical Topical Meetings on CD-ROM (The Optical Society of America, Washington, DC, 2006).

2. **Mengna Xia**, Vikram Kodibagkar, Ralph Mason, Benjamin Levine, Hanli Liu, "Tumor vascular and tissue oxygenation dynamics under normobaric and hyperbaric oxygen interventions", presented at the fourth Era of Hope meeting for the Department of Defense (DOD) Breast Cancer Research Program (BCRP) held on June 8-11, 2005 in Philadelphia, Pennsylvania.

3. Xiufeng Li, **Mengna Xia**, Edmond Richer, Matthew Lewis, Billy Smith, Ammar Adam, Ralph Mason, Hanli Liu, Peter P. Antich, "Optical Imaging Phantom Study for Quantitative Imaging Correction and Physiological Parameter Detection in vivo, Molecular Medicine Symposium", presented in the University of Texas System: Translating Discoveries into Health, Houston, TX, 2005.

4. **Mengna Xia**, Ralph Mason, Hanli Liu, "A model of hemodynamic responses of rat tumors to hyperoxic gas challenge", Proc. SPIE- Optical Tomography and Spectroscopy of Tissue VI, 5693: 301-307 (2005).

**Manuscripts in preparation:**

1. Megan Xiao, Ralph Mason, Benjamin Levine, and Hanoi Liu, "Tumor Vascular and tissue oxygen dynamics simultaneously monitored by NIRS and fluorescence oxygen sensor in rats under hyperbaric oxygen exposure," to be submitted to *the Open Biomedical Engineering*.

2. Mengna Xia, Ralph Mason, Benjamin Levine, and Hanli Liu, "Tumor vascular oxygenation monitored by NIRS in rats with hyperbaric oxygen intervention in combination with doxorubicin treatment." to be submitted to *Breast Cancer Research*.

## 4. Conclusions

In the last year's study, we have learned that HBO enhances the therapeutic action of doxorubicin in our tumor model, probably by multiple physiological mechanisms. The present study reveals that DOX may be used in conjunction with HBO to obtain the same effect as higher doxorubicin doses. Meanwhile, NIRS may work as an attractive approach to monitor the cardiotoxicity of treatment.

Overall, our study over the last few years has demonstrated that 1) NIRS and MRI and FOXY$^{TM}$ oxygen sensor are complimentary approaches to monitor tumor oxygenation. 2) Tumor tissue oxygenation achieved by hyperbaric oxygenation persists over 10-20 minutes even after terminating hyperbaric oxygenation intervention. 3) Several correlations were existed for both modalities under sequences of hyperoxic gas intervention with hyperbaric oxygen exposure. Correlation of tumor vascular oxygenation and tumor tissue $pO_2$ determined by those techniques simultaneously could give us a better understanding on the patho-physiology of tumor and response to therapeutic interventions. 4) HBO enhanced the therapeutic action of doxorubicin in our tumor model, probably by multiple physiological mechanisms. DOX could be used in conjunction with HBO to achieve the same effect as higher doxorubicin doses. NIRS may work as an attractive approach to monitor the cardiotoxicity of treatment.

## 5. References


1 . Weiss, R., G. Sarosy, K. Clagett-carr, M. Russo, and B. Leyland-jones, *Anthracyline analogs: the past, present, and future.* Cancer Chemother Pharmacol, 1986. **18**: p. 185-97.

2. Brown, J.M., *The hypoxic cell: a target for selective cancer therapy--eighteenth Bruce F. Cain Memorial Award lecture.* Cancer Res., 1999. **59**(23): p. 5863-5870.

3. Hall, E., *Radiobiology for the Radiologist*. 4th ed. 1994, Philadelphia, PA: Lippincott.

4. Thews, O., D. Kelleher, and P. Vaupel, *Erythropoietin restores the anemia-induced reduction in cyclophosphamide cytotoxicity in rat tumors.* Cancer Res, 2001. **61**: p. 1358-61.

5. Bush, R., R. Jenkin, W. Allt, F. Beale, A. Dembo, and J. Pringle, *Definitive evidence for hypoxic cells influencing cure in cancer therapy.* Br J Cancer, 1978. **37**: p. 302-06.

6. Hall, E.J., *The oxygen effect and reoxygenation,* in *Radiobiology for the Radiologist,* E.J. Hall, Editor. 1994, J. B. Lippincott: Philadelphia. p. 133-152.

7. Brady, L., H. Plenk, J. Hanley, J. Glassburn, S. Kramer, and R. Parker, *Hyperbaric oxygen therapy for carcinoma of the cervix stages IIB, IIIB, and IVA: results of a randomized study by the radiation therapy oncology group.* Int J Radiat Onclo Biol Phys, 1981. **7**: p. 991-8.

8. Brizel, D., W. Hage, R. Dodge, M. Munley, C. Piantadosi, and M. Dewhirst, *Hyperbaric Oxygen Improves Tumor Radiation Response Significantly More Than carbogen/Nicotinarnid.* Radiation Res., 1997. **147**: p. 715-20.

9. Rockwell, S., M. Kelley, C. Irvin, C. Hughcs, E. Porter, H. Yabuki, and J. Fischer, *Modulation of tunlor oxygenation and radiosensitivity by a perfluorooctylbromide emulsion.* Radiother Oncol, 1991. **22**: p. 92-8.

10. Lustig, R., N. Lowe, C. Rose, J. Haas, S. Krasnow, M. Spaulding, and I. Prosnitz, *Phase 1/11 study kluosol and 100% oxygen as an adjuvant to radiation i11 the treatment of locally advanced non-small cell carcinoma of the head and neck.* Int. .I. Radiat. Oncal. Biol. Phys., 1989. **16**: p. 1587-93.

11. Xia, M., V. Kodibagkar, R. Mason, B. Levine, and H. Liu. Tumor vascular and tissue oxygenation dynamics under normobaric and hyperbaric oxygen interventions. in fourth Era of Hope meeting for the Department of Defense (DOD) Breast Cancer Research Program (BCRP). 2005. Philadelphia, PA.

12. Kalns, J., L. Krock, and E. Piepmeier, *The effect of hyperbaric oxygen on growth and chemosensitivity of metastatic prostate cancer.* Anticancer Res, 1998. **18**.

13. Petre, P., F. Baciewicz, S. Figan, and J. Spear, *Hyperbaric oxygen as a chemotherapy adjuvant in the treatment of metastatic lung tumors in a rat model.* J Thorac Cardiovasc Surg, 2003. **125**: p. 85-95.

14. Kalns, J., L. Krock, and E. Piepmeier, *The effect of hyperbaric oxygen on growth and chemosensitivity of metastatic prostate cancer.* Anticancer Res, 1998. **18**.

15. Takiguchi, N., N. Saito, M. Nunomura, K. Kouda, K. Oda, N. Furuyama, and N. Nakajima, *Use of 5-FU plus hyperbaric oxygen for treating malignant tumors:evaluation of antitumor effect and measurement of 5-FU in individual organs.* Cancer Chemother Pharmacol, 2001. **47**: p. 11-14.

16. Stuhr, L., Hyperbaric oxygen alone or combined with 5-FU attenuates growth of DMBA-induced rat mammary tumors. Cancer Lett, 2004. **210**: p. 35-40.

17. Zhao, D., L. Jiang, and R. Mason, *Measuring changes in tumor oxygenation.* Methods Enzymol, 2004. **386**: p. 378-418.

18. Jobsis, F., *Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters.* Science, 1977. **198**: p. 1264-7.

19. Liu, H., Y. Song, K.L. Worden, X. Jiang, A. Constantinescu, and R.P. Mason, *Noninvasive Investigation of Blood Oxygenation Dynamics of Tumors by Near-Infrared Spectroscopy. Appl. Opt.*, 2000. **39**(28): p. 5231-5243.

20. Delpy, D.T. and M. Cope, *Quantification in tissue near-infrared spectroscopy.* Phil. Trans. R. Soc. Lond. B., 1997. **352**: p. 649-659.

21. Homma, S., T. Fukunaga, and A. Kagaya, Influence of adipose tissue thickness on near-infrared spectroscopic signals in the measurement of human muscles. J. Biomed. Opt., 1996. **1**: p. 418-424.

22. Xia, M., V. Kodibagkar, H. Liu, and R.P. Mason, Tumour oxygen dynamics measured simultaneously by near-infrared spectroscopy and 19F magnetic resonance imaging in rats. Physics in medicine and biology, 2006. **51**: p. 45-60.
23. Kim, J., D. Zhao, R. Mason, and H. Liu. Acute effects of combreatastatin A4 phosphate on breast tumor hemodynamics monitored by Near Infrared Spectroscopy. in in Biomedical Optics 2006 Technical Digest (Optical Society of America, Washington, DC, 2006). 2006.

24. Kim, J., D.J. Cuccia, J. Lee, A.E. Cerussi, A.J. Durkin, and B.J. Tromberg. Monitoring of chemotherapy effects on rat breast tumors: a correlation between optical signals and histology. in SPIE. 2007. San Jose, CA.

25. Cohen, M., A. Johnston-Early, M. Hood, M. McKenzie, M. Citron, N. Jaffe, and S. Krasnow, *Drug precipitation within i.v. tubing: a potential hazard of chemotherapy administration.* Cancer Treat Rep. 1985 Nov;69(11):1325-6, 1985. **69**: p. 1325-6.

26. Esenaliev, R., Y. Petrov, O. Hartrumpf, D. Deyo, and D. Prough, Continuous, noninvasive monitoring of total hemoglobin concentration using an optoacoustic technique. Appl Opt, 2004. **43**: p. 3401-07.

27. Cope, M., *the application of near infrared spectroscopy to non invasive monitoring of cerebral oxygenation in the newborn infant*. 1991, Ph.D dissertation in University of London.

28. Alagoz, T., R. Buller, B. Anderson, K. Terrell, R. Squatrito, T. Niemann, D. Tatman, and P. Jebson, *Evaluation of hyperbaric oxygen as a chemosensitizer in the treatment of epithelial ovarian cancer in xenografts in mice.* Cancer, 1995. **75**: p. 2313-22.

29. Kong, Q., J. Beel, and K. Lillehei, *A threshold concept for cancer therapy.* Med Hpyotheses, 2000. **55**: p. 29-35.

30. Harrisona, L., M. Chadhaa, R. Hillb, K. Hua, and D. Shashaa, *Impact of tumor hypoxia and anemia on radiation therapy outcomes.* Oncologist, 2002. **7**: p. 492-508.

31. Kizaka-Kondoh, S., M. Inoue, H. Harada, and M. Hiraoka, *Tumor hypoxia: a target for selective cancer therapy.* Cancer Sci, 2003. **94**: p. 1021-28.

32. McMillan, T., K. Calhoun, J. Mader, C. Stiernberg, and S. Rajaraman, *The effect of hyperbaric oxygen on oral mucosal carcinoma.* Laryngoscope, 1989. **99**: p. 241-44.

33. Kalns, J. and E. Piepmeier, *Exposure to hyperbaric oxygen induces cell cycle perturbation in prostate cancer cells.* In Vitro Cell Dev Biol Anim, 1999. **35**: p. 98-101.

34. Murata, T., H. Yamawaki, M. Hori, K. Sato, H. Ozaki, and H. Karaki, *Chronic vascular toxicity of doxorubicin in an organ-cultured artery.* British J of Pharm, 2001. **132**: p. 1365-73.

35. Kanmura, Y., L. Raeymaekers, and R. Casteels, *Effects of doxorubicin and ruthenium red on intracellular Ca2+ stores in skiined rabbit mesenteric smooth-muscle fibres.* Cell Calcium, 1989. **10**: p. 433-39.

36. Ferrans, V., J. Clark, J. Zhang, Z. Yu, and E. Herman, *Pathogenesis and prevention of doxorubicin cardiomyopathy.* Tsitologiia, 1997. **39**: p. 928-37.

**ORGANIZATION: The University of Texas at Arlington** **DATE:09/05/07**

# REPRESENTATIONS FOR ASSISTANCE AGREEMENTS

**1. TYPE OF BUSINESS ORGANIZATION**

The offeror, by checking all applicable boxes, represents that it operates as:

____X_____ an Educational Institution (__X__ state-controlled or ____ private)
____X_____ a Nonprofit Organization
_____ a For-Profit Organization
_____ an Historically Black College or University
_____ a Minority Institution
_____ a Small Business
_____ a Large Business

**2. AUTHORIZED NEGOTIATORS**

The offeror or quoter represents that the following persons are authorized to negotiate on its behalf with the Government in connection with this request for proposals or quotations:
James Spaniolo, President, Dr. Ronald Elsenbaumer, Interim Provost, Dr. Kelsey Downum, Interim Vice President for Research, Jeremy Forsberg, Director, Research Administration
(817) 272-2105 Fax (817) 272-5808 email ogcs@uta.edu

(list names, titles, and telephone and FAX numbers of the authorized negotiators).

**3. DUNS NUMBER**

The offeror is requested to provide the 9-digit DUNS number on the following line:

DUNS Number: 06-423-4610

If the offeror does not have a DUNS number, go to website: http://www.dnb.com.

**4. TAXPAYER IDENTIFICATION**

Definitions.

"Common parent," as used in this solicitation provision, means that corporate entity that owns or controls an affiliated group of corporations that files its Federal income tax returns on a consolidated basis, and of which the offeror is a member.

"Corporate status," as used in this solicitation provision, means a designation as to whether the offeror is a corporate entity, an unincorporated entity (e.g., sole proprietorship or partnership), or a corporation providing medical and health care services.

"Taxpayer Identification Number (TIN)," as used in this solicitation provision, means the number required by the IRS to be used by the offeror in reporting income tax and other returns.

All offerors are required to submit the information required in paragraphs (c) through (e) of this solicitation provision in order to comply with reporting requirements of 26 U.S.C. 6041, 6041A, and 6050M and comply with reporting requirements of 26 U.S.C. 6041, 6041A, and 6050M and implementing regulations issued by the Internal Revenue Service (IRS). If the resulting award is subject to the reporting requirements described in FAR 4.903, the failure or refusal by the offeror to furnish the information may result in a 20 percent reduction of payments otherwise due under the award.

Taxpayer Identification Number (TIN)

/x_/ TIN: 75-6000121

/_/ TIN has been applied for

/_/ TIN is not required because:

/_/ Offeror is a nonresident alien, foreign corporation, or foreign partnership that does not have income effectively connected with the conduct of a trade or business in the U.S. and does not have an office or place of business or a fiscal paying agent in the U.S.;

/_/ Offeror is an agency or instrumentality of a foreign government;

/_/ Offeror is an agency or instrumentality of a Federal, state, or local government;

/_/ Other. State basis.

Corporate Status

/_/ Corporation providing medical and health care services, or engaged in the billing and collecting of payments for such services;

/_/ Other corporate entity;

/_/ Not a corporate entity;

/_/ Sole proprietorship

/_/ Partnership

/_/ Hospital or extended care facility described in 26 CFR 501(c)(3) that is exempt from taxation under 26 CFR 501(a).

Common Parent

/_/ Offeror is not owned or controlled by a common parent as defined above.

/_/ Name and TIN of common parent:

Name

TIN

**5. INSTITUTION CODE**

The Offeror is requested to provide its Federal Interagency Committee on Education (FICE) Institution Code on the following line:

Institution Code: 003656

**6. COMMERCIAL AND GOVERNMENT ENTITY (CAGE) CODE REPORTING**

The Offeror is requested to enter its CAGE code on the following line and on its offer in the block with its name and address. The CAGE code entered must be for that name and address. Enter CAGE before the number.

CAGE Code: 2N798

If the Offeror does not have a CAGE code, go to website: http://www.dlis.dla.mil/cageserv.asp and follow the instructions given.

The offeror should not delay submission of the offer pending receipt of a CAGE code.

**7. RESPONSIBILITY - PERFORMANCE RECORD**

Pre-award Survey Information: The Grants Officer must make a determination of a recipient's responsibility prior to awarding a grant or cooperative agreement. The offeror shall complete the following to facilitate this determination.

(a) Yes (x ) No ( ) This organization will be able to accomplish the objectives of the research contained in the schedule. This statement is taking into consideration all existing business commitments, commercial as well as Governmental.

(b) A minimum of two current references (preferably Governmental) for whom contracts, grants or cooperative agreements for same/similar items identified in this proposal have been satisfactorily completed.

| | |
|---|---|
| Department Of Energy | Department of Energy |
| NAME OF AGENCY | NAME OF AGENCY |
| DE-FG02-06ER64284 09/01/06 | |
| Award NO. DATE | |
| | DE-FG02-04ER15623 09/15/04 |
| | AWARD NO. DATE |
| $753,000 | $303,649 |
| AMOUNT | AMOUNT |
| The University of Texas at Arlington Multimodel Optical Research Section of AIRC | Spatially Directed and Photoassisted Electrosynthesis of Semiconductors and Nanocomposites |
| TITLE OF RESEARCH | TITLE OF RESEARCH |
| Dr. Arthur Katz | |

| | |
|---|---|
| | Dr. Mark Spitler |
| GRANTS OFFICER'S NAME | GRANTS OFFICER'S NAME |
| 301-903-4932 | 301-903-4568 |
| TELEPHONE NO. AND AREA CODE | TELEPHONE NO. AND AREA CODE |

**8. AWARD/PAYMENT ADDRESS**

In the event the offeror is awarded an agreement, the offeror shall indicate below the **award address:**

701 S. Nedderman
Box 19145
Arlington, Texas 76019-0145

The offeror shall indicate below the address to which any payments should be mailed if that address is different from the award address shown above.

**Payment address:**

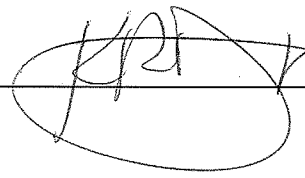
219 W. Main
Arlington, Texas 76010-0136

**9. AUTHORIZATION TO PERFORM**

The Recipient represents that it has been duly authorized to operate and to do business in the country or countries in which this award is to be performed. The Recipient also represents that it will fully comply with all laws, decrees, labor standards, and regulations of such country or countries, during the performance of this award.

**10. FEDERAL DEMONSTRATION PARTNERSHIP (FDP) STATUS**

The recipient represents that it is ( X ), is not (  ) an active member eligible to receive an award under the FDP. Information on the FDP is located at http://www.TheFDP.org.

Signature  Date 9/5/07

Typed/Printed Name: Dr Kelsey Downum  Title: Interim Vice President for Research